CPA
Accredited Medical Laboratory
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The Newcastle upon Tyne Hospitals NHS
NHS Foundation Trust

USER MANUAL for
Newcastle Mitochondrial NSCT Diagnostic Service

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Newcastle University, Framlington Place, Newcastle upon Tyne, NE2 4HH
Telephone: 0191-2824375 **Fax:** 0191-2824373 www.mitochondrialncg.nhs.uk

General Laboratory Information

Location: Main laboratory Room M4012, Medical School
Main Office Room M4028, Medical School

Service Hours: Monday – Friday: 08:00 to 17:00

Head of Laboratory: Professor Rob Taylor (Tel: 0191-2223685 direct line)
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Clinical Service (Adults): Professor Doug Turnbull (Tel: 0191-2824375)
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Dr Andrew Schaefer (Tel: 0191-2824375)
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Clinical Service (Children): Dr Robert McFarland (Tel: 0191-2824375)
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Clinical Scientists: Dr Emma Watson (Tel: 0191-2228877)
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Dr Steven Hardy (Tel: 0191-2228877)
Miss Jessica Gabriel (Tel: 0191-2228877)

Biomedical Scientists: Mr Gavin Falkous (Tel: 0191-2228877)
Mrs Angela Baker (Tel: 0191-2228877)

Genetic Technologist: Dr Kate Hickman (Tel: 0191-2228877)
Mrs Karen Baty (Tel: 0191-2228877)

Medical Laboratory Assistant: Mr Aaron Potts (Tel: 0191-2228877)

Quality Manager: Mr Alan Smith (Tel: 0191-2418715)
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Service Administrator: Mrs Jane Brown (Tel: 0191-2824375)
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Background Information to the Service

Mitochondria are ubiquitous organelles that contain their own genetic complement, the mitochondrial genome (mtDNA). Although intimately involved in many cellular processes, their principal task is to provide the energy necessary for normal cell functioning and maintenance. Disruption of this energy supply can have devastating effects for the cell, organ and individual. One important consequence of mitochondrial ubiquity is that mitochondrial disease can affect virtually any organ and present with a plethora of symptoms and signs to a variety of specialties. These are truly multi-system diseases with significant morbidity and mortality. Over the last two decades, mutations in both mtDNA and nuclear DNA (nDNA) have been identified as causative in a number of the mitochondrial clinical syndromes, although for mtDNA mutations in particular, this relationship between genotype and phenotype is often far from straightforward. A number of epidemiological studies have been undertaken to assess the prevalence of mitochondrial disease and whilst rare these conditions have a major impact on both the community and individual families. Finally, there is increasing awareness by clinicians experienced in the management of patients with mitochondrial disease that many aspects of mitochondrial disease can be helped or prevented by early diagnosis and subsequent care.

The Newcastle Mitochondrial Diagnostic Laboratory is an affiliated laboratory of the Northern Regional Genetics Service, and is situated within the research laboratory space of the University's Mitochondrial Research Group in the Medical School. In partnership with teams at Queen Square, Institute of Neurology (UCLH NHS Foundation Trust) and the Oxford Medical Genetics Laboratories, Churchill Hospital (Oxford Radcliffe Hospitals NHS Trust), the Newcastle lab has been awarded National Specialist Commissioning (NSCT) funding to provide a comprehensive, diagnostic and clinical management service for patients with mitochondrial disease in England and Scotland – "***Rare Mitochondrial Disorders Service for Adults and Children***". This multi-disciplinary service will encompass all aspects of diagnosis (muscle biopsy, histochemistry, biochemistry and molecular genetics), utilising specialist clinical and laboratory skills available at the three component centres, and complementing the routine analysis of common mtDNA mutations that is available throughout the UK at many regional genetics laboratories. For many patients with suspected mitochondrial disease, it is not possible to make a diagnosis based solely on molecular genetic testing in blood DNA, and so for those patients where more detailed investigations are required, a comprehensive diagnostic service is now offered which combines clinical investigations, histochemical and histological analysis of patient muscle biopsies, measurement of respiratory chain complex activities together with screening of both mtDNA and nuclear-encoded mitochondrial genes.

Services offered by the Newcastle Mitochondrial NSCT Laboratory

CLINICAL SERVICES

Outpatient

Consultations are available for patients and families with suspected or proven mitochondrial disease. Clinical examination may confirm the suspicion of mitochondrial disease or suggest an alternative diagnosis. Clear plans of investigation or patient management will be established during the clinic appointment with close collaboration between the referring consultant and the patients own general practitioner. Whenever possible, follow up will be at local hospitals, although some patients with particularly rare conditions or where local services are limited may require longer term follow up in Newcastle.

Consultations are also available for women with proven mtDNA disease or who are carriers of pathogenic mtDNA mutations and who require advice concerning the possible transmission of the mtDNA mutation to their children. Patients or families in which mitochondrial respiratory chain

disease is confirmed but in whom the genetic diagnosis has not been established, or with mutations in known nuclear genes, also have access to this service.

Inpatient
Day-case and inpatient services are available for patients who have to travel for specific diagnostic investigations, and are available for both children and adults. Muscle biopsies are not always available in certain hospitals and these can be arranged to be performed in Newcastle as part of the diagnostic work-up.

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E-mail: catherine.feeney@nuth.nhs.uk or catherine.feeney@nhs.net

Secretaries: Sue Callender (sue.callender@nuth.nhs.uk or susan.callender@nhs.net)
Bernadette Caygill (bernadette.caygill@nuth.nhs.uk or bernadette.caygill@nhs.net)

For clinicians wishing to refer patients for a clinical opinion (either as outpatients or inpatients), please write to Professor Turnbull (adult referrals) or Dr McFarland (paediatric referrals) at the following address:

Newcastle Mitochondrial NSCT Laboratory
Wellcome Trust Centre for Mitochondrial Research,
4th Floor Cookson Building
The Medical School
Newcastle University
Framlington Place
Newcastle upon Tyne
NE2 4HH

Tel. 0191-2824375 Fax. 0191-2824373
E.mail: jane.brown@nuth.nhs.uk or jane.brown38@nhs.net

LABORATORY SERVICES

Histological and Histochemical analysis of muscle biopsy samples
An unfixed frozen muscle sample, in transverse orientation, measuring approximately **3mm x 3mm x 3mm (25 mg) is the minimum** required for mitochondrial histochemical analysis, which will include cytochrome *c* oxidase (COX) staining, succinate dehydrogenase (SDH) staining and sequential COX-SDH assays to thoroughly investigate the possibility of low levels of COX-deficient fibre. As appropriate in specific cases, other tissues are accepted for the investigation of mitochondrial histochemical activities including heart (e.g. suspected mitochondrial cardiomyopathies) and liver (e.g. Alpers' Syndrome). Details for the dispatch of frozen tissues are given later in this manual.

Respiratory chain enzyme measurements in muscle biopsy samples
To fully assess mitochondrial respiratory chain activity in frozen tissue samples, we assay the activities of respiratory chain complexes I, II, III and IV individually and compare these activities to the activity of the mitochondrial matrix marker enzyme citrate synthase, an indicator of mitochondrial mass within the sample.

An unfixed, snap-frozen muscle sample is required for these investigations, preferably unmounted and free from OCT and cork. Ideally, between **100-150 mg muscle tissue** is preferred to be able to perform these assays with **60 mg tissue the absolute minimum amount** of sample we will accept.

Molecular genetic analysis for suspected mitochondrial disorders
The laboratory offers a range of tests for both mtDNA mutations, ranging from point mutation analysis to whole mitochondrial genome sequencing, and the analysis of several nuclear genes involved in mtDNA maintenance. For many common mtDNA point mutations, genetic screening can be undertaken using blood DNA, although the use of alternative sources such as urinary cell sediment DNA samples are recommended for some mutations, in particular the m.3243A>G mutation. For the study of possible mtDNA rearrangements and whole mitochondrial genome, muscle DNA is essential.

Samples for genetic analysis:

Muscle: If a muscle biopsy is already being referred for either histochemical or biochemical assessment, we are able to extract DNA either directly from cryostat-cut sections or from a residual, nuclear pellet generated during the preparation of an enriched mitochondrial fraction for enzyme studies. The details for dispatch of muscle biopsy samples on dry ice are given later in this manual.

Blood: Extracted DNA or 2-3 x 4.5ml EDTA-blood (purple top) tubes should be sent for analysis; please use EDTA as anticoagulant rather than heparin, which precludes molecular genetic testing. Mix the blood well after taking, but do not freeze; these samples can be stored at 4°C for 1-2 days if required without problems, and should be shipped at room temperature.

Urine: Urine samples (40-50ml) should be collected into sterile universal or falcon-type tubes and forwarded to the lab at room temperature; do not freeze. To ensure the best possible yield of epithelial cells from these samples, an early morning urine sample (first pass) is preferable.

Other tissues: Other tissue samples can be accepted for DNA analysis, including pathology blocks, cultured cells, buccal mouth scrapes and hair samples. The investigation of several, non-invasive DNA samples can be particularly useful in determining the pathogenic behaviour of specific (or novel) mtDNA mutations. Please contact the laboratory for specific instructions as to what we require and how to send these samples.

Please forward the samples to:

Professor Rob Taylor, Newcastle Mitochondrial NSCT Laboratory
Wellcome Trust Centre for Mitochondrial Research, 4th Floor Cookson Building, The Medical School, Newcastle University, Framlington Place, Newcastle upon Tyne, NE2 4HH

Blood or any fluid samples should be in an appropriate container, wrapped in absorbent material, and placed inside a sturdy plastic container (e.g. biobottle) for transport.

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MUSCLE BIOPSY - DISPATCH INSTRUCTIONS

NOTE: We are not able to accept samples designated, or suspected, as being "High Risk" (i.e. from patients with HIV, hepatitis, CJD, polio etc)

1. **Please inform the Service Administrator or laboratory of your intention to send a sample on dry ice before it leaves your laboratory, either by phone or by e-mail. Please note, we cannot accept responsibility for the safety of samples sent without the prior knowledge of our unit.**

2. When you send the samples, please include a covering letter with the names and addresses of any additional people who will require copies of letters or reports. More clinical information is always helpful, in addition to that provided on the referral form. Please send a copy of the muscle histopathology report, if available.

3. The muscle sample must be sent in dry ice, using a recognised courier (TNT or FedEx) for 'door to door' delivery. A "next day before 12 noon" delivery service should be used. Please ensure that sufficient dry ice is used to last 48 hours (at least 10kg, but dependent on size of polystyrene container). Ensure that no absorbent material is insulating the primary vessel from the dry ice. Do not use polystyrene chips or freezing blocks to fill the polystyrene container.

4. Address the package to:

   Professor Rob Taylor
   Newcastle Mitochondrial NSCT Laboratory
   Wellcome Trust Centre for Mitochondrial Research,
   4th Floor Cookson Building
   The Medical School
   Newcastle University
   Framlington Place
   Newcastle upon Tyne
   NE2 4HH

   It is helpful to place address labels on more than one side of the box because the courier usually puts their own label on top.

5. Please try and send samples on a Monday, Tuesday or Wednesday. Do not send samples to arrive on a Friday, since delays may well move the delivery of your package into the weekend. Our unit is not staffed at these times and your sample will thaw and be lost.

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Current list of tests available at the Newcastle Mitochondrial NSCT Diagnostic Laboratory

Please contact the lab directly if further details are required.

HISTOLOGICAL/HISTOCHEMICAL ANALYSES

Each frozen sample is assessed for the following:

- H&E staining of cryostat-cut sections
- Cytochrome *c* oxidase (COX) activity
- Succinate dehydrogenase (SDH) activity
- Sequential COX-SDH activities
- Modified Gomori trichrome staining

MITOCHONDRIAL RESPIRATORY CHAIN ANALYSES

Each frozen sample is assessed for the following:

- Complex I (NADH:ubiquinone oxidoreductase) activity
- Complex II (Succinate:ubiquinone oxidoreductase) activity
- Complex III (Ubiquinol:cytochrome *c* oxidoreductase) activity
- Complex IV (Cytochrome *c* oxidase) activity
- Citrate Synthase

MOLECULAR GENETIC ANALYSES

MUTATION/DISORDER TO BE TESTED	TYPE OF TEST/ SAMPLE NEEDED
m.3243A>G point mutation (MELAS syndrome, maternally-inherited diabetes and deafness (MIDD))	Pyrosequencing to allow estimation of mtDNA heteroplasmy. Blood and/or urine DNA; muscle if available
m.8344A>G, m.8356T>C and m.8363G>A mutations (MERRF syndrome)	sequencing of entire *MTTK* ($tRNA^{Lys}$) gene for known pathogenic mutations. Blood DNA or muscle if available
m.8993T>G/C and m.9176T>G/C mutations (NARP and MILS (maternally-inherited Leigh Syndrome)	sequencing of the mitochondrial *MTATP6* and *MTATP8* genes. Blood DNA appropriate
LHON mutations	screening of 3 primary LHON mutations (m.3460G>A, m.11778G>A and m.14484T>C) using blood DNA

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MUTATION/DISORDER TO BE TESTED	TYPE OF TEST/ SAMPLE NEEDED
mtDNA rearrangement disorders Kearns-Sayre, Pearsons syndrome)	Long-range PCR and/or Southern (CPEO, blotting of affected tissues to investigate single mtDNA and secondary multiple mtDNA deletions. Muscle DNA essential, blood DNA for children with Pearsons syndrome
Mitochondrial deafness mutations (*MTRNR1* (12S rRNA) gene including m.1555A>G mutation and *MTTS1* ($tRNA^{Ser(UCN)}$ gene)	Sequencing of both genes. Blood DNA samples appropriate for this analysis
***MTND* gene sequencing** (patients with isolated complex I deficiency or LHON patients in whom common mutations previously excluded	Sequencing of all mitochondrial-encoded complex I genes. DNA from affected tissue (e.g. muscle) essential in cases of complex I deficiency; patients with LHON can be assessed in blood.
***MTCYB* gene sequencing** (patients with isolated complex III deficiency)	Sequencing of mitochondrial-encoded cytochrome *b* gene. DNA from affected tissue (e.g. muscle) essential
Mitochondrial genome sequencing (Screen for rare or novel pathogenic mtDNA mutations)	DNA from affected tissue (e.g. muscle) essential
Single fibre real-time PCR (Quantification and screening of mtDNA rearrangements in individual muscle fibres (COX-deficient and COX-positive) by real-time PCR	An unfixed frozen muscle biopsy sample, in transverse orientation to allow histochemical analysis and isolation of single cells by laser microdissection
mtDNA depletion syndromes (Assessment of mtDNA copy number to screen for possible mtDNA depletion syndromes)	Taqman® real-time PCR assay which coamplifies nuclear (18S rRNA) and mtDNA (*MTND1*) genes. DNA from affected tissues (e.g. muscle, liver) essential
***POLG1* mutation screening** (*POLG1* mutations cause a phenotypic spectrum ranging from severe encephalopathy and liver failure typical of Alpers syndrome to late-onset PEO, ataxia, myopathy and epilepsy associated with multiple mtDNA deletions and depletion)	Sequencing of the entire *POLG1* coding region. Blood DNA samples appropriate and required for complete gene analysis
***PEO1* (Twinkle)** Patients with dominant/recessive PEO and multiple mtDNA deletions	Sequencing of the entire *PEO1* coding region. Blood DNA samples appropriate and required.

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MUTATION/DISORDER TO BE TESTED	TYPE OF TEST/ SAMPLE NEEDED
***SLC25A4* (ANT1)** Patients with dominant PEO and multiple mtDNA deletions	Sequencing of the entire *SLC25A4* coding region. Blood DNA samples appropriate and required.
POLG2 Patients with dominant PEO and multiple mtDNA deletions	Sequencing of the entire *POLG2* coding region. Blood DNA samples appropriate and required.
TK2* and *RRM2B Patients with dominant/recessive PEO and multiple mtDNA deletions	Sequencing of the entire *TK2*/*RRM2B* coding regions. Blood DNA samples appropriate and required .
TK2* and *RRM2B Patients with evidence of mtDNA depletion in skeletal muscle	Sequencing of the entire *TK2* and *RRM2B* coding regions. Blood DNA samples appropriate and required.
DGUOK* and *MPV17 Patients with evidence of mtDNA depletion in liver	Sequencing of the entire *DGUOK* and *MPV17* coding regions. Blood DNA samples appropriate and required.
SUCLA2* and *SUCLG1 Patients with evidence of mtDNA depletion and methylmalonic aciduria	Sequencing of the entire *SUCLA2* and *SUCLG1* coding regions. Blood DNA samples appropriate and required.
TRMU Patients with evidence of acute liver disease and respiratory chain deficiency	Sequencing of the entire *TRMU* coding region. Blood DNA samples appropriate and required.
DARS2 Patients with mitochondrial leukoencephalopathy, brain stem and spinal cord involvement, lactic acidosis (LBSL)	Sequencing of the entire *DARS2* coding region. Blood DNA samples appropriate and required.
RARS2 Patients with multiple respiratory chain complex deficiencies and pontocerebellar hyoplasia	Sequencing of the entire *RARS2* coding region. Blood DNA samples appropriate and required.
AARS2 Patients with multiple respiratory chain complex deficiencies and hypertrophic cardiomyopathy	Sequencing of the entire *AARS2* coding region. Blood DNA samples appropriate and required.
Nuclear complex I structural genes A panel of 10 genes can be screened in patients with isolated complex I deficiency (*NDUFV1*, *NDUFV2*, *NDUFS1*, *NDUFS2*, *NDUFS3*, *NDUFS4*, NDUFS6, *NDUFS7*, *NDUFS8*, *NDUFAB1*)	Sequencing of the entire coding region of nine structural genes. Blood DNA samples appropriate and required.

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***ACAD9* – complex I assembly factor** Patients with isolated complex I deficiency; often associated with cardiac phenotype	Sequencing of the entire coding region. Blood DNA samples appropriate and required.
Complex II structural and assembly genes Patients with evidence of isolated complex II deficiency. Sequencing of the four structural genes (*SDHA*, *SDHB*, *SDHC*, *SDHD*) and known assembly factors (*SDHAF1* and *SDHAF2*)	Sequencing of the entire coding regions. Blood DNA samples appropriate and
Nuclear complex III genes Patients with isolated complex III deficiency are screened for mutations in the *BCS1L* gene, a known complex III assembly factor	Sequencing of the entire coding region. Blood DNA samples appropriate and required.
TMEM70, ATPAF2* and *ATP5E Patients with suspected complex V deficiency	Sequencing of the entire coding regions. Blood DNA samples appropriate and required.
AGK Patients with multiple respiratory chain complex deficiencies and Sengers syndrome (cataracts, HCM and elevated lactate)	Sequencing of the entire *AGK* coding region. Blood DNA samples appropriate and required.
NFU1 Patients with multiple respiratory chain complex deficiencies (complex I, III and III) – FeS cluster scaffold gene defect	Sequencing of the entire *NFU1* coding region. Blood DNA samples appropriate and required.
MTO1 Patients with multiple respiratory chain complex Deficiencies, hypertrophic cardiomyopathy and and elevated lactate	Sequencing of the entire *MTO1* coding region. Blood DNA samples appropriate and required.

Targeted Haloplex Panels for Nuclear complex I structural genes and mtDNA maintenance genes – Please contact Professor Taylor to discuss

CVB and prenatal testing for nuclear and mtDNA mutations	Please contact Professor Taylor to discuss individual cases – please note that there is a charge applicable for this testing as this is not covered under our NSCT agreement.
PGD for specific mtDNA mutations	Please contact Professor Taylor and Dr McFarland to discuss individual cases - please note that there is a charge applicable for this testing as this is not covered under our NSCT agreement.

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Sample Reporting Times

Enzyme histochemistry	Within 2 weeks
Respiratory chain enzyme analysis	Within 4-6 weeks (***please contact the laboratory if urgent analysis is required***)
Urgent molecular diagnostic tests	Within 7 working days (PCR based)
CVB analysis	24-48 hour turnaround
Non-urgent samples	Within 4-8 weeks
Mitochondrial genome sequencing	8-10 weeks
***POLG1* sequencing**	common mutations screened within 4 weeks; Whole gene: 8-12 weeks
Nuclear maintenance gene analysis	If relevant, these will be assessed sequentially with the first gene reported within an 8 week timeframe
Nuclear complex I gene analysis	8-12 weeks for full panel of nine genes
Other nuclear genes	Within 4-8 weeks

External Quality Control

We participate in the following external quality assessment schemes.

UKNEQAS for Molecular Genetics (Mitochondrial Disorders)
European Molecular Genetics Quality Network (Molecular Genetic Sequencing Scheme)
UKNEQAS for Cytopathology Technique

There are currently no external schemes available in the UK for mitochondrial respiratory chain enzyme analysis, but we have developed an annual sample exchange programme with other NCG service laboratories including Professor Simon Heales/Dr Iain Hargreaves lab at UCLH and Dr Garry Brown's lab at Oxford.

User Satisfaction Survey for Laboratory Services

As part of our quality management system and to ensure that we are meeting the needs of our users, we are always keen to receive any comments you may have on the quality of the service we provide and would welcome any suggestions on ways in which we might be able to improve the service.

Please feel free to contact the laboratory Quality Manager (alan.smith@nuth.nhs.uk) with any suggestions; an electronic questionnaire is available directly by email should this be required.

CPA Accreditation Status

The Newcastle Mitochondrial Diagnostic Service Laboratory is accredited by Clinical Pathology Accreditation (UK) Ltd and operates a quality management system in accordance with CPA Standards for the Medical Laboratory.

CPA reference 3118

United Kingdom Genetic Testing Network

The Newcastle Mitochondrial Diagnostic Service Laboratory is registered with UKGTN as a provider of genetic testing for mitochondrial diseases